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Water Stability Characteristic of Nile Tilapia (*Oreochromis niloticus*) Feed Coated with Tapioca Flour-beeswax-based Edible Coating

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Abstract

One of the reasons for the increasing water turbidity in Nile tilapia aquaculture is the presence of fish feed spoilage that easily crumbles and decomposes, releasing organic matter into the water. To address this issue, a thin layer or edible coating is needed to cover the tilapia feed, which would slow down the decomposition caused by water absorption. The main coating ingredients include tapioca flour combined with beeswax solution and glycerol as a plasticizer. In this study, three different glycerol concentrations were used: 3% w/v, 5% w/v, and 7% w/v, along with three beeswax concentrations: 0.25% w/v, 0.50% w/v, and 0.75% w/v. The primary objective of this study was to analyze the effect of these plasticizers and beeswax on the characteristics of the edible coating. The dipping method was employed to coat the tilapia feed, wherein the product is dipped in the coating solution. To assess the results, functional group tests were conducted using FTIR spectra, contact angle tests, and water resistance tests of the coated fish feed. The longest sinking time was observed when tilapia feed was coated with a solution containing 0.75% w/v beeswax and 3% w/v glycerol.

Keywords: Beeswax, edible coating, fish feed, nile tilapia, tapioca

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) production in Indonesia tends to rise from 1,249,060.05 tons in 2018 to 1,337,831.69 tons in 2019 (Kementerian Kelautan dan Perikanan, 2022). The increase in production is due to the characteristics of tilapia, which are easy to cultivate, omnivorous, adaptive, and have a high tolerance for various environmental conditions. The availability and sustainability of feed are part of the main factors in intensive or semi-intensive tilapia culture. Hence commercial feed is preferable to fresh one.

Pellet is one of the most common types of fish feed. The pellet that quickly breaks down into smaller particles and leaches nutrients can reduce water quality in the culture environment (Obirikorang, Amisah, Fialor, & Skov, 2015). The accumulation of organic compounds from feed causes a reduction in oxygen levels and increases the toxic substances. These effects lead to a decline in fish growth and influence the survival rate of tilapia. Therefore, the water stability of tilapia feed should be improved.

As one of the physical characteristics of fish feed, water stability is influenced by composition in food formulation. Polysaccharides have a role as binding agents in feed formulation. Previous studies have explained the water stability of fish pellets by adding several starch sources, as demonstrated by Solomon, Ataguba, & Abeje (2011), Onada & Ogunola (2020), and Orire & Emine (2019). A combination of cassava tuber starch and *Saccharomyces cerevisiae* or brewer yeast as a floatation agent to the feed shows that feed has 50 to 60 minutes of durability in water (Solomon, Ataguba, & Abeje, 2011; Onada & Ogunola, 2020). Moreover, cassava starch alone is recommended to increase the pellet's float ability to 100% for 60 minutes in water (Orire & Emine, 2019). Furthermore, non-starch binders, including plant-origin and microbial-origin gums, also improve pellet durability in water (Brown, 2015; Saleela, Somanath, & Palavesam, 2015). An alternative option is adding pellets with fats, like fish, stearin, coconut, and hazelnut oils supplements, in formulation for rising water stability of feed around

60 to 120 minutes (Haetami, Junianto, Iskandar, Rostika, & Abun, 2017; Guo, Davis, Starkey, & Davis, 2021).

In general, manufacturing floating pellets requires a complex process, especially during gelatinization. (Hakim, Handoyo, Novianto, & Prasetyo, 2019). Instead of mixing a binder agent to increase the water resistance of fish feed, another simple method is to coat the fish feed with a hydrophobic edible coating. This method allows fish farmers to modify commercial feed independently, according to their needs. Edible coating preparations, compositions, and production techniques have been broadly employed in food and animal feed (Vonasek, Choi, Sanchez, & Nitin, 2018; Huang & Nitin, 2019). For example, an edible coating made from fermented soy pulp has been applied to fish feed pellets, affecting the floatation and water stability of the feed. (Zulhisyam, Kabir, Munir, & Wei, 2020).

Recently, the literature needs to explain the wettability of fish feed and the consequences of water stability. The physical properties of the feed play a key role in the water's durability feature. Hence, this study focuses on the physicochemical properties of the edible coating and its application on tilapia feed. It aims to get the best edible coating formulation to increase the pellet's water stability and float ability. A facile and simple method has been proposed for producing edible coating using tapioca flour incorporated with various concentrations of beeswax as matrix components. The additives, namely plasticizer, and emulsifier, are added to enhance the physical characteristics of the edible coating (Bertuzzi & Slavutsky, 2016).

METHODOLOGY

Materials and Instrumentals

Tapioca flour was purchased in the local market in Balikpapan, while food-grade beeswax was ordered from Bandung. Glycerol (local) was chosen as a plasticizer. Then, the emulsifier was tween 80 (local) bought from Surabaya.

Methods

Tapioca flour solution was made with a ratio of tapioca flour and distilled water as 1: 10, then heated using a hotplate at a temperature of 100˚C and stirred at 800 rpm for 1 hour. Next, a glycerol solution was prepared by dissolving 3%, 5%, and 7% w/v of glycerol in 10 mL of distilled water. After that, a mixture of beeswax solutions with an emulsifier, namely tween 80, was prepared by mixing 40 ml of distilled water with 1 ml of tween 80. Then, beeswax is added with a variation of 0.25; 0.5; 0.75 % w/v. The three components were homogenized by heating the solution using a hotplate at 120°C and stirring at 800 rpm for 30 minutes. The beeswax-emulsifier mixture was then added to the tapioca solution. The homogenization of the coating solutions was carried out by heating at a temperature of 100˚C and stirring at a speed of 1200 rpm for 30 minutes. After 30 minutes, the solutions were cooled at room for 20 minutes to reduce their temperature. Then, the fish feed is dip-coated into the coating solutions, followed by a drying process for 2 hours 40 minutes at 105°C.

In this study, the effects of various concentrations of beeswax on the water stability of fish feed are observed. To prepare the beeswax solution, the emulsifier plays a crucial role in dispersing the beeswax. Additionally, various concentrations of plasticizers are inspected to achieve optimal results.

Three tests were conducted to characterize the coated fish feed, namely functional group analysis, wettability, and water stability tests. The functional group analysis was conducted at the Laboratorium Mineral dan Material Maju, Universitas Negeri Malang, using Fourier Transform Infrared Spectroscopy (FTIR). This analysis gives the compositions of the edible coating to prove that all the materials have been homogenized. The wettability test was carried out by coating the glass slide with a coating solution. Then, the dry film was dropped with 20μL of water, and the drop profile was observed using a camera. The contact angle was determined by ImageJ software. Then, the water stability test was conducted by soaking the coated feed for 24 hours in water. The observation was done until the fish feed sank and the water became turbid.

Data Analysis

The water stability results were analysed by ANOVA, specifically by Tukey's Method, using Minitab 19.

RESULTS AND DISCUSSION

Functional group analysis

The result of the functional group analysis is presented in Figure 1, where the coating solution's functional group shows an absorption band at 2916.41 cm-1, indicating the presence of a C-H bond within the frequency range of 3000-2850 cm-1. This C-H bond is characteristic of an aldehyde group, which has been detected in starch. Furthermore, the aldehyde group is bound within the glucose structure. Glucose serves as a monomer of amylose and amylopectin, both of which are integral components of starch. In the edible coating, amylose contributes to the compactness of the coating, while amylopectin enhances its stability (Suarni & Widowati, 2005).

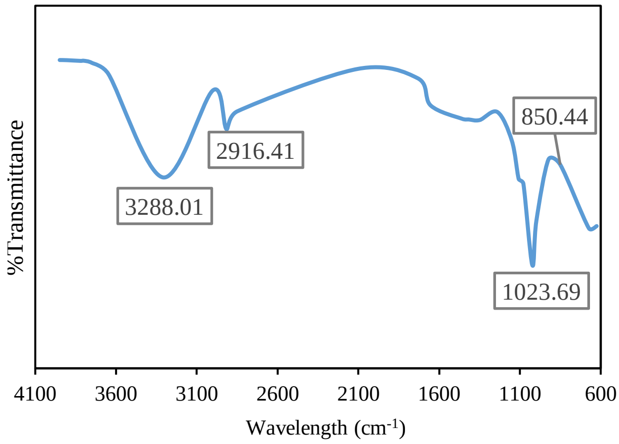

Figure 1. FTIR spectra of edible coating

The absorption band at 3308.68 cm^{-1} indicates the presence of an O-H bond with a free -H bond. The intermolecular O-H bond in the starch chain is broken due to the presence of the O-H bond in glycerol. This effect causes the straight chains of starch (amylose and amylopectin) to break and influences the dried coating preparation process (Murni, Pawignyo, Widyawati, & Sari, 2015). The detected functional groups are summarized in Table 1. Its results suggesting that the coating solution has been well dispersed.

Table 1. Characteristics of FTIR spectra

Types of Vibration	Wavelength (cm^{-1})
O-H	3288.01
C-H	2931.59
C-O	1016.84
C-H aromatic	757.36

The edible coating consists of starch, which comprises amylose and amylopectin components. Therefore, starch contains C-H bonds, C-O bonds, O-H bonds, and aromatic C-H bonds. The FTIR spectra indicate that the manufacturing of edible coating involves a physical mixing process, facilitated by the presence of hydrogen bonds between the chains. Based on the results of the functional group analysis, it can be inferred that the detected functional groups are composed of those from starch, glycerol, and beeswax.

Wettability Result

Edible coating without the addition of glycerol does not exhibit contact angles. The coating is rigid, leading to its breakage and scattering when applied to a glass slide. These phenomena can be attributed to the absence of elastic properties in the edible coating solution. According to Sarkar, Ravi, and Alexandridis 2013, an increase in glycerol concentration reduces the cohesion bonds between polymers, resulting in a more elastic film. The hydroxyl group of glycerol can form hydrogen bonds, replacing the polymer-polymer bonds in the coating. Glycerol is commonly used as a plasticizer because it is compatible with amylose, which promotes better mechanical properties in the coating. Additionally, glycerol can reduce the intermolecular forces between starch molecules (Nordin, Othman, Rashid, & Basha, 2020). Glycerol's low molecular weight allows it to penetrate the polysaccharide matrix, thus increasing flexibility (Bergo & Sobral, 2007). According to Yulianti & Ginting, 2012, glycerol in a water-starch mixture can reduce the stress value. These properties of glycerol contribute to its role in improving the elasticity and mechanical performance of the edible coating.

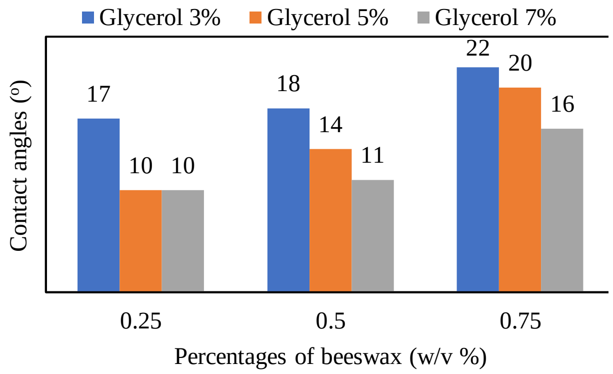

Figure 2. Relation between contact angle and beeswax-glycerol concentrations.

Figure 2 illustrates the relationship between beeswax-glycerol concentrations and contact angle. Higher concentrations of beeswax are associated with greater contact angle values. This finding aligns with the results of Santoso (2006), which suggest that increasing the concentration of beeswax reduces the rate of water vapor transmission due to the hydrophobic properties of wax. On the other hand, increasing the concentration of glycerol leads to smaller contact angles. This can be attributed to the fact that higher glycerol concentrations result in increased water vapor absorption (Wahyu, Sitompul, & Zubaidah, 2017; Ningsih, Ariyani, & Sunardi, 2019).

When beeswax is added to the coating, it produces a film with a larger contact angle. This is because the adhesion force produced by the film is reduced with the addition of beeswax, leading to a larger contact angle. The adhesion force between the solid and the liquid affects the contact angle, where a greater adhesion force corresponds to a smaller contact angle (Fritzsche & Peuker, 2014). The highest contact angle value was obtained at the variable of 0.75% w/v beeswax with 3% w/v glycerol, which was measured at 22 degrees. Figure 3 displays a comparison of contact angles at various beeswax concentrations combined with 3% w/v glycerolwith 3% w/v of glycerol is shown in Figure 3.

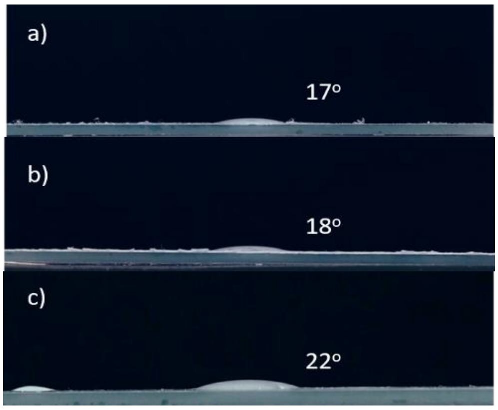

Figure 3. The contact angle of (a) 3% w/v of glycerol and 0.25% w/v of beeswax, (b) 3% w/v of glycerol and 0.5% w/v of beeswax, (c) 3% w/v of glycerol and 0.75% w/v of beeswax.

Water Stability Result

Based on the wettability test results, the water stability of fish feed coated with various concentrations of beeswax and glycerol was analyzed. Figure 4 displays the water stability test of the fish feed coated with 0.75% w/v beeswax and 3% w/v glycerol. As observed, increasing the concentration of beeswax tends to reduce the solubility of the edible coating, making the coating less polar. This low polarity of beeswax is beneficial as it allows the feed to float longer in water and prevents rapid decomposition. In summary, the results indicate that the incorporation of beeswax and glycerol in the edible coating positively affects the water stability of fish feed. The reduced solubility and increased hydrophobic nature of the coating facilitate its ability to remain buoyant in water for an extended period, providing better water stability for the coated fish feed.

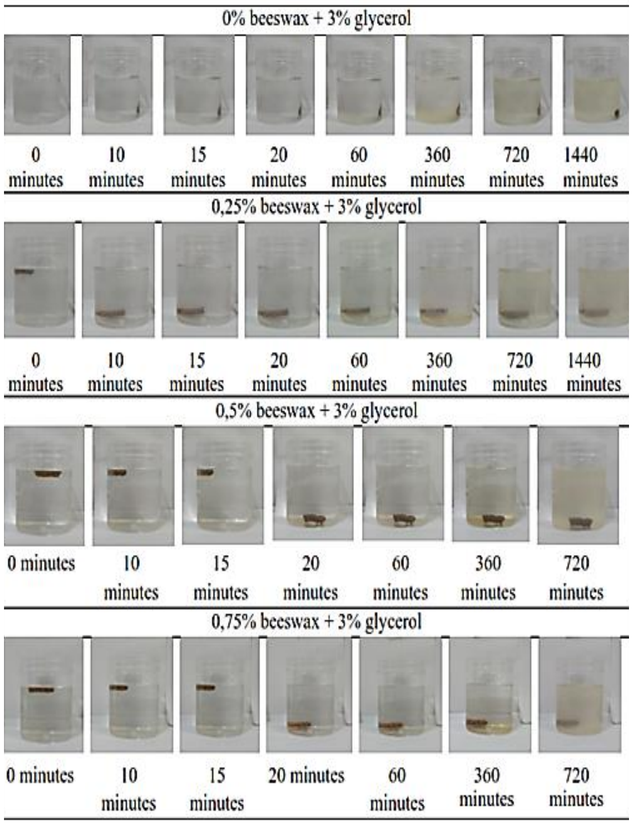

Figure 4. Results of water stability test of tilapia fish feed coated with 3% w/v of glycerol and various concentrations of beeswax.

Data Analysis

Based on the statistical results, significant differences in sinking time are indicated by different letters, as shown in Table 2. The ANOVA results confirm that the variation in beeswax concentration has a significant influence on the water stability of fish feed, while the different concentrations of glycerol at the same beeswax concentration do not have a noticeable effect on water stability. As a result, there is no significant difference in sinking time between the various glycerol concentrations within each beeswax concentration group.

The sinking time of fish feed is directly proportional to the increase in beeswax concentrations in the coating solution. This correlation is consistent with the hydrophobic nature of beeswax, as previously shown in the study by Oliveira, Santos, Leite, Aroucha, & Silva (2018). Considering material efficiency and cost-effectiveness, the composition of 0.75% w/v beeswax and 3% w/v glycerol is considered the best treatment for enhancing the water stability of fish feed. In conclusion, the statistical analysis shows that beeswax concentration significantly affects the water stability of fish feed, while glycerol concentration does not have a noticeable impact. The use of 0.75% w/v beeswax and 3% w/v glycerol in the coating solution is recommended as the most efficient and cost-effective treatment for improving the water stability of fish feed.

Table 2. Statistical result of water stability of coated fish feed.

Beeswax (%w/v)	Glycerol (%w/v)	N	Sinking time (sec)
0.75	7	3	1340.00[a]
0.75	5	3	1148.67[ab]
0.75	3	3	1099.00[ab]
0.5	7	3	976.00[b]
0.5	5	3	943.00[b]
0.5	3	3	919.67[b]
0.25	7	3	478.00[c]
0.25	5	3	443.33[c]
0.25	3	3	440.33[c]

CONCLUSION

The combination of beeswax and glycerol as additives in tapioca flour-based coating solution, has improved the water stability of nile tilapia fish feed. It is shown that hydrophobicity of beeswax affects the sinking time of coated fish feed. The suggested composition for coating solution is tapioca flour and water with ratio of 1:10, 0.75% w/v of beeswax, and 3% w/v of glycerol.

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